REMARKS

The Examiner is respectfully requested to enter this Reply After Final in that it raises no new issues. Alternatively, the Examiner is respectfully requested to enter this Reply After Final in that it places the application in better form for Appeal.

Status of the Claims

Claims 5-9 are pending in the present application. Claims 1-4 have been canceled. Claims 6-9 have been added. Claim 5 has been amended to recite that the claimed assay is an immunoassay of human medullasin. No new matter has been added by way of the above amendments. In addition no new issues have been raised by the claim amendments. As such, Applicants respectfully request that the amendments be entered.

Rejection under 35 USC 103(a)

The Examiner maintains the rejection of claims 1-5 as obvious over Aoki et al. in view of Kohler. Applicants traverse the rejection and respectfully request the withdrawal thereof.

Present Invention

The present invention is directed to an immunoassay for human medullasin in the blood using an anti-human medullasin

monoclonal antibody. Specifically, the immunoassay for human medullasin in the blood uses an anti-human medullasin monoclonal antibody immobilized on an insoluble carrier and a labeled anti-human medullasin monoclonal antibody, to capture the human medullasin in the test blood sample on the inert carrier by forming a sandwich-structured complex with the human medullasin by way of the antigen-antibody reactions, and quantifies the label in the complex.

The anti-human medullasin monoclonal antibody originates from the mouse and is obtained by culturing hybridomas prepared by cell fusion between antibody-producing cells recovered from the mouse immunized with human medullasin and myeloma cells, to specifically recognize the human medullasin recovered from the culture.

Moreover, the present invention, as demonstrated by Example 2, has a one hour time period (the total time required for the incubations, 30 minutes for the first incubation and 30 minutes for the second incubation) to measure the medullasin. The results produced by the short assay are highly accurate. See for example, the calibration chart of Figure 1, which shows a well-correlated relationship with concentration, scattering to only a limited extent.

Distinguishing the Present Invention

Aoki et al. discloses an enzyme-aided immunoassay for quantitatively analyzing human medullasin in the blood by the aid of a polyclonal antibody (See page 195). Aoki et al. uses a polyclonal antibody for the quantitative analysis of human medullasin in the blood. The method disclosed in Aoki takes a total of long 18 hours to complete, as described in Clinica Chimika Acta, volume 178, the last 2 lines on page 195 to line 11 in page 196. Two hours is required for the first incubation step for reacting IgG immobilized on polystyrene beads with medullasin in the blood, and 16 hours is required for the second incubation step for the medullasin polyclonal antibody conjugate.

Aoki et al. fails to disclose or suggest substituting a monoclonal antibody with a polyclonal antibody. Aoki et al. also fails to disclose or suggest shortening the incubation times for arriving at the present invention. Please see the attached chart entitled, "Comparison with the Present Invention and Aoki et al." for a summary of these arguments.

Kohler discloses a basic process for producing monoclonal antibodies. Kohler does not disclose or suggest using a monoclonal antibody in an immunoassay as recited in the present claims.

A polyclonal antibody is a mixture of antibodies of varying

reactivity, some are high and the others low, and the immunoassay of human medullasin by the polyclonal antibody needs a long reaction time because of a high proportion of antibodies of low reactivity contained in the polyclonal antibody. By contrast, the monoclonal antibody the inventors of the present invention use is an individual monoclonal antibody of very high reactivity, and can determine medullasin content in a very short time.

The difference between a polyclonal and monoclonal antibody in reactivity greatly depends on the antigen for which it is used, and it is a peculiar phenomenon that a monoclonal antibody is much more reactive with medullasin than a polyclonal antibody.

Neither Kohler nor Aoki discloses substituting monoclonal antibodies for the assay disclosed in Aoki. Furthermore, neither Kohler nor Aoki discloses arriving at the present invention of an immunoassay that produces results within one hour.

No Prima Facie Obviousness

Applicants submit that the Examiner has failed to establish a prima facie case of obviousness, because the Examiner has failed to show that one of ordinary skill in the art would be motivated to combine the teachings of Aoki et al with Kohler to arrive at an immunoassay that is capable of producing results within one hour and that uses monoclonal antibodies.

In the absence of some teaching to combine the references or that there is some likelihood of arriving at the present invention from a combination of the two references, Applicants submit that the Examiner should withdraw the obviousness rejections in view of the above remarks.

Conclusion

As Applicants have addressed and overcome all rejections in the Office Action, Applicants respectfully request that the rejections be withdrawn and that the claims be allowed.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Kecia Reynolds (Reg. No. 47,021) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

Attached hereto is a marked-up version of the changes made to the application by this Amendment.

Pursuant to the provisions of 37 C.F.R. § 1.17 and 1.136(a), Applicants hereby petition for an extension of two (2) months to October 15, 2002 for the period in which to file a response to the outstanding Office Action. The required fee of $400.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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Attachment: Table for Comparison
Version with Markings to Show Changes Made

(Rev. 02/20/02)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend the claims as follows:

5. (Amended) An immunoassay of a human medullasin comprising immobilizing human medullasin in a test sample by sandwiching said human medullasin between an anti-human medullasin monoclonal antibody immobilized on an insoluble carrier and a labeled anti-human medullasin monoclonal antibody by antigen-antibody reactions to form a complex, and quantifying said label in said complex.

Claims 6-9 are added.

Comparison with the present invention and Aoki et al

	The present invention Example 2(3)	Aoki et al
I	In a test tube, one beads on which a mouse anti-human medullasin monoclonal antibody(2E04) was immobilized, 50μl of 2% BSA-containing PBS solution containing purified human medullasin(standard substance) in a concentration of 0, 1, 10, 100 or 200 ng/ml, and 350μl of 2% BSA-containing 0.2μg/ml HRP-labeled the mixture of incubated at 37°C for **30minutes.**	Polystyrene balls coated with IgG were incubated with various amounts of medullasin or peripheral blood diluted with 0.1mol/l sodium phosphate buffer, pH6.5, at 37°C for **2h** with shaking in a final volume of 0.15ml.
II	After removing the contents of the test tube by aspiration, the test tube was washed with physiological saline and 400μl of 0.1 M phosphate citrate buffer(pH4.6) containing 0.05% ABTS and 0.034% hydrogen peroxide was added to each test tube. The mixture was incubated at 37°C for **30minutes.** After that 1ml of 0.1N aqueous oxalic acid solution wad added to each test tube to stop the enzyme reaction and the absorbance at 420 nm of the resulting solution was meassured with a spectrophotometer.	After incubation polystyrene balls were washed twice with 1ml of buffer A without NaN_3 and incubated with 50ng of the conjugate in a final volume of 0.15ml A without NaN_3 at 25°C for **16h** with shaking.